

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

1-40. (Cancelled)

41. (Currently amended) A method of diagnosing a human pregnant subject as having, or having a propensity to develop, pre-eclampsia or eclampsia, said method comprising measuring the level of sFlt-1 polypeptide in a sample from said subject, wherein a level of sFlt-1 polypeptide greater than 2 ng/ml diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

42. (Currently amended) A method of diagnosing a human pregnant subject as having, or having a propensity to develop, pre-eclampsia or eclampsia, said method comprising measuring the level of free PlGF polypeptide in a serum sample from said subject, wherein said free PlGF is a PlGF polypeptide that has the ability to bind to sFlt-1, and wherein said subject is pregnant and a level of free PlGF polypeptide less than 150 pg/ml serum at ~~13-16~~ less than 16 weeks of pregnancy diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

43. (Cancelled)

44. (Currently amended) A method of diagnosing a human pregnant subject as having, or having a propensity to develop, pre-eclampsia or eclampsia, said method comprising measuring the level of free VEGF polypeptide in a sample from said subject, wherein said free VEGF is a VEGF polypeptide that has the ability to bind to sFlt-1, and wherein said subject is pregnant and a level of free VEGF polypeptide less than 5 pg/ml serum diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

45. (Currently amended) A method of diagnosing a human pregnant subject as having, or having a propensity to develop, pre-eclampsia or eclampsia, said method comprising measuring the levels of at least two of sFlt-1, free VEGF, and free PlGF polypeptide in a sample from said subject, wherein said free VEGF is a VEGF polypeptide that has the ability to bind to sFlt-1 and wherein said free PlGF polypeptide is a polypeptide that has the ability to bind to sFlt-1, and comparing the level to the level of at least two of sFlt-1, free VEGF, or free PlGF polypeptide in a reference, and wherein an increase of at least 10% in the level of sFlt-1 or a decrease of at least 10% in the level of free VEGF or free PlGF polypeptide relative to said reference diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

46. (Previously presented) The method of claim 45, wherein said method comprises measuring the level of sFlt-1 and at least one of free VEGF and free PlGF, and wherein said method further comprises calculating the relationship between said level of sFlt-1 and said at least one of free VEGF and free PlGF using a metric, wherein an increase of at least 10% in the level of said sFlt-1 relative to at least one of said free VEGF and free PlGF level in said metric from said subject sample as compared to said metric from a reference sample diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

47. (Currently amended) The method of claim 46, wherein said metric comprises a pre-eclampsia anti-angiogenic index (PAAI):
$$\text{PAAI} = \frac{\text{sFlt-1}}{\text{free VEGF} + \text{free PlGF}}$$
, and an increase of at least 10% in said PAAI in said subject sample as compared to said reference ~~is a diagnostic indicator of pre-eclampsia or eclampsia~~ diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

48. (Currently amended) A method of diagnosing a human pregnant subject as having, or having a propensity to develop, pre-eclampsia or eclampsia, said method comprising:

(a) measuring the levels of sFlt-1, free VEGF, and free PlGF polypeptides in a sample from a subject, wherein said free VEGF is a VEGF polypeptide that

has the ability to bind to sFlt-1 and wherein said free PlGF polypeptide is a polypeptide that has the ability to bind sFlt-1; and

(b) calculating the relationship between said levels of sFlt-1, free VEGF, and free PlGF using a metric, wherein said metric is comprises a pre-eclampsia anti-angiogenic index (PAAI):[sFlt-1/ free VEGF + free PlGF], and wherein a PAAI value greater than 20 in the subject sample ~~is a diagnostic indicator of pre-eclampsia or eclampsia~~ diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

49. (Currently amended) The method of claim 46, wherein said metric comprises sFlt-1/free PlGF and an increase of at least 10% in the sFlt-1/free PlGF from said subject sample as compared to said reference ~~is a diagnostic indicator of pre-eclampsia or eclampsia~~ diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

50. (Currently amended) A method of diagnosing a human pregnant subject as having, or having a propensity to develop, pre-eclampsia or eclampsia, said method comprising measuring the level of at least one of sFlt-1, free VEGF, or free PlGF polypeptide in a sample from a subject, wherein said free VEGF is a VEGF polypeptide that has the ability to bind to sFlt-1 and wherein said free PlGF polypeptide is a polypeptide that has the ability to bind sFlt-1, and comparing the level to the level of sFlt-1, free VEGF, or free PlGF polypeptide in a reference,

and wherein an increase of at least 10% in the level of sFlt-1 or a decrease of at least 10% in the level of free VEGF or free PlGF polypeptide relative to said reference diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

51-53. (Canceled)

54. (Previously presented) The method of claim 46, 47, or 49, wherein said metric further comprises the body mass index or gestational age of the subject.

55. (Previously presented) The method of claim 45, 46, 47, 49, or 50 wherein said reference is a prior sample or level from said subject.

56. (Previously presented) The method of claim 45, 46, 47, 49, or 50, wherein said reference is a sample taken from a control subject not having pre-eclampsia or eclampsia.

57. (Canceled)

58. (Currently amended) The method of claim 41, 44, 45, 46, 48, 49, or 50, wherein said subject is in the first trimester of pregnancy.

59. (Currently amended) The method of claim 41, 44, 45, 46, 48, 49, or 50, wherein said subject is in the second trimester of pregnancy.

60. (Currently amended) The method of claim 41, 44, 45, 46, 48, 49, or 50, wherein said subject is in the third trimester of pregnancy.

61. (Currently amended) The method of claim 41, 44, 45, 46, 48, 49, or 50, wherein said subject is ~~13~~less than 16 weeks pregnant.

62. (Currently amended) The method of claim 41, 42, ~~43~~, 44, 45, 48, or 50, wherein said measuring is done using an immunological assay.

63. (Previously presented) The method of claim 62, wherein said immunological assay is an ELISA.

64. (Currently amended) The method of claim 41, 44, 45, 46, 48, or 50, wherein said sample is a bodily fluid of said subject in which said sFlt-1, free VEGF, or free PlGF polypeptide is normally detectable.

65. (Currently amended) The method of claim 64, wherein said bodily fluid is selected from the group consisting of urine, ~~amniotic fluid~~, serum, and plasma.

66. (Previously presented) The method of claim 45 or 50, wherein said sample is a cell or a tissue from said subject.

67. (Previously presented) The method of claim 66, wherein said tissue is a placental tissue.

68. (Currently amended) The method of any one of claims 45, 47, 49, or 50, wherein said subject is further diagnosed as having, or having a propensity to develop, mild pre-eclampsia, severe pre-eclampsia, or pre-eclampsia-associated HELLP, IUGR, or SGA.

69. (Cancelled)

70. (Currently amended) The method of claim 41, 45, 48, 49, or 50, wherein said sFlt-1 is the level of free sFlt-1.

71. (Currently amended) The method of claim 41, 45, 48, 49, or 50, wherein said sFlt-1 is the level of bound sFlt-1.

72. (Currently amended) The method of claim 41, 45, 48, 49, or 50, wherein said sFlt-1 is the level of total sFlt-1.

73. (Previously presented) The method of claim 45 or 50, wherein an increase of at least 50% in the level of sFlt-1 or a decrease of at least 50% in the level of free VEGF or free PlGF polypeptide relative to said reference diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

74. (Previously presented) The method of claim 73, wherein an increase of at least 90% in the level of sFlt-1 or a decrease of at least 90% in the level of free VEGF or free PlGF polypeptide relative to said reference diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

75. (Previously presented) The method of claim 47, wherein an increase of at least 50% in said PAAI in said subject sample as compared to said reference is a diagnostic indicator of pre-eclampsia or eclampsia.

76. (Previously presented) The method of claim 75, wherein an increase of at least 90% in said PAAI in said subject sample as compared to said reference is a diagnostic indicator of pre-eclampsia or eclampsia.

77. (Currently amended) The method of claim 49, wherein an increase of at least 50% in said sFlt-1/free PlGF in said subject sample as compared to said reference ~~is a diagnostic indicator of pre-eclampsia or eclampsia~~ diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

78. (Currently amended) The method of claim 77, wherein an increase of at least 90% in said sFlt-1/free PlGF in said subject sample as compared to said reference ~~is a diagnostic indicator of pre-eclampsia or eclampsia~~ diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

79. (Currently amended) The method of claim 42-~~or~~ 43, said method further comprising measuring the level of sFlt-1 in said subject sample, wherein a level of sFlt-1 polypeptide greater than 2 ng/ml diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

80. (Previously presented) The method of claim 45, said method comprising measuring the levels of sFlt-1 and free PlGF polypeptides.

81. (Currently amended) The method of claim 66, wherein said cell is selected from the group consisting of an endothelial cell, a leukocyte, ~~a monocyte,~~ and a cell derived from the placenta.

82. (New) A method of diagnosing a human pregnant subject as having, or having a propensity to develop, pre-eclampsia or eclampsia, said method comprising measuring the level of free PlGF polypeptide in a serum sample from said subject and the level of sFlt-1 in a bodily fluid sample from said subject, wherein said free PlGF is a polypeptide that has the ability to bind to sFlt-1, and wherein a level of free PlGF polypeptide less than 400 pg/ml serum at mid-gestation and wherein a level of sFlt-1 polypeptide greater than 2 ng/ml diagnoses said subject as having, or having a propensity to develop, pre-eclampsia or eclampsia.

83. (New) The method of claim 44, 45, 48, or 50, wherein said VEGF polypeptide is selected from the group consisting of VEGF-A, VEGF-B, VEGF189, VEGF165, and VEGF121.

84. (New) The method of claim 42, 45, 48, 49, 50, or 82, wherein said PlGF polypeptide is an alternatively spliced isoform of PlGF.

85. (New) The method of claim 81, wherein said leukocyte is a monocyte.

86. (New) The method of claim 45, wherein the level of sFlt-1 and free PlGF are measured and wherein said subject is less than 16 weeks pregnant.

87. (New) The method of claim 45, wherein the level of sFlt-1 and free PlGF are measured and wherein said subject is 17-20 weeks pregnant.

88. (New) The method of claim 41, 45, 49, 50, 80, or 82, wherein said diagnosis is prior to the development of at least one symptom of pre-eclampsia or eclampsia in said subject, said at least one symptom selected from the group consisting of a systolic blood pressure (BP) >140 mmHg and a diastolic BP >90 mmHg after 20 weeks gestation; new onset proteinuria; greater than 300 mg of protein in a 24-hour urine collection; and a single random urine sample having a protein/creatinine ratio greater than 0.3.

89. (New) The method of claim 41, 42, 45, 48, 49, or 50, wherein the method diagnoses said pregnant human subject as having a propensity to develop pre-eclampsia or eclampsia.

90. (New) The method of claim 41, 42, 45, 48, 49, or 50, wherein the method diagnoses said pregnant human subject as having pre-eclampsia or eclampsia.

91. (New) The method of claim 45, 50, or 80, wherein the subject is 23-32 weeks pregnant and an increase of at least 50% in the level of sFlt-1 or a decrease of at least 50% in the level of free VEGF or free PlGF relative to said reference diagnoses said subject as having a propensity to develop early onset pre-eclampsia or eclampsia.